cSN50 Peptide

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Peptide-directed Suppression of a Pro-inflammatory Cytokine Response*

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Signal-dependent nuclear translocation of transcription factor nuclear factor κB (NF-κB) is required for the activation of downstream target genes encoding the mediators of immune and inflammatory responses. To inhibit this inducible signaling to the nucleus, we designed a cyclic peptide (cSN50) containing a cell-permeable motif and a cyclized form of the nuclear localization sequence for the p50-NF-κB1 subunit of NF-κB. When delivered into cultured macrophages treated with the pro-inflammatory agonist lipopolysaccharide, cSN50 was a more efficient inhibitor of NF-κB nuclear import than its linear analog. When delivered into mice challenged with lipopolysaccharide, cSN50 potently blocked the production of proinflammatory cytokines (tumor necrosis factor α and interferon γ) and significantly reduced the lethality associated with ensuing endotoxic shock. Based on specificity studies conducted with a mutated form of cSN50, a functional nuclear localization motif is required for this protective effect. Taken together, our findings demonstrate effective targeting of a cell-permeable peptide that attenuates cytokine signaling *in vivo*. This new class of biological response modifiers may be applicable to the control of systemic inflammatory reactions.

Signal transduction and gene transcription in response to proinflammatory agonists lead to rapid expression of genes encoding mediators of inflammatory and immune reactions (1, 2). Three of the key mediators of these localized and systemic responses are the cytokines, tumor necrosis factor α (TNFα),[1] interferon γ (IFNγ), and interleukin 1 (IL-1). The genes encoding these cytokines are activated in response to inflammatory, immune, and oxidative stress via a mechanism involving nuclear translocation of stress-responsive transcription factors (SRTFs). As exemplified by NF-κB and NFAT, SRTFs are sequestered in the cytoplasm and mobilized to the nucleus following cellular stimulation (3–6). In turn, SRTFs interact with specific cognate sites present in the promoter region of genes encoding mediators of inflammatory and immune responses. For example, the gene encoding TNFα is regulated by NF-κB, NFAT, and ATF2/Jun, whereas the gene encoding IFNγ is controlled by NF-κB and NFAT (7–9).

Cytokine-mediated systemic inflammatory response can be evoked by lipopolysaccharide (LPS) (10–13). In response to LPS, monocytes, macrophages, and endothelial cells deploy SRTFs to their nuclear compartments (14–18). Persistent nuclear translocation of NF-κB in human and mouse mononuclear phagocytic cells correlates with the lethal outcome of systemic inflammatory response syndrome (19). Thus, development of this syndrome involves a multiplex process that is triggered by mobilization of NF-κB and other SRTFs to their nuclear sites of action and by systemic expression of proinflammatory cytokine mediators.

TNFα and IFNγ play a key role in the systemic inflammatory response syndrome induced by LPS, which has lethal effects in experimental animals. In keeping with this concept, animals deficient in receptors for these cytokines are resistant to lethal endotoxic shock (11, 20, 21). Thus, controlling the expression of these cytokine genes could provide an opportunity to ameliorate the ensuing systemic inflammatory responses. Considering the regulatory linkage between SRTFs and cytokine gene expression, we developed a method for noninvasive, intracellular delivery of peptides that interfere with SRTF signaling (for review see Ref. 22). The cell-permeable SN50 peptide we designed for these studies carries the hydrophobic region (h region) of the signal peptide as a membrane translocating motif and a nuclear localization sequence (NLS) derived from the p50-NF-κB1 subunit of transcription factor NF-κB. This linear peptide inhibits the nuclear import of NF-κB in human monocytic cells and murine endothelial cells stimulated with the proinflammatory agonists LPS and TNFα (23). The SN50 peptide also inhibits the inducible nuclear import of AP-1, NFAT, and signal transducers and activators of transcription 1 (24, 25).

In terms of its mechanism of action, we have demonstrated in prior studies that the SN50 peptide interacts *in vitro* with a cytoplasmic NLS receptor comprised of the Rch 1/importin (karyopherin)-β heterodimer (24). Peptide-directed interference with nuclear import of SRTFs in cultured cells attenuates the inducible expression of cyclooxygenase 2 (COX 2) protein (26) and the level of mRNA transcripts of the IL-2 and Fas ligand genes (24, 27). These data indicate that SN50 affects downstream SRTF-regulated genes. In this report, we demonstrate that *in vivo* delivery of a more potent cyclic analog

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[1] The abbreviations used are: TNFα, tumor necrosis factor α; LPS, lipopolysaccharide; NLS, nuclear localization sequence; NF-κB, nuclear factor κB; IFNγ, interferon γ; AP-1, activator protein 1; NFAT, nuclear factor of activated T cells; SRTF(s), stress-responsive transcription factor(s); IL, interleukin; COX 2, cyclooxygenase 2; MEM, minimal essential medium; FACS, fluorescence-activated cell sorting; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; cSN50, cyclic SN50.

TABLE I
Sequences of cSN50, SN50, and SM peptides

Sequences are given in single letter amino acid code. Cell membrane-permeable sequence (*underlined*) is derived from the hydrophobic (h) domain of the Kaposi fibroblast growth factor signal sequence, and NLS is derived from NF-κB1 (23). Peptides were synthesized and purified as described previously (23, 24, 25).

Peptide	Cell-permeable sequence NLS motif or its mutant
cSN50 peptide	AAVALLPAVLLALLAPCYVQRKRQKLMPC
FITC-cSN50 peptide	(FITC)GGGAAVALLPAVLLALLAPCYVQRKRQKLMPC
SN50 peptide	AAVALLPAVLLALLAPVQRKRQKLMP
SM peptide	AAVALLPAVLLALLAPAAADQNQLMP

(cSN50) of this cell-permeable inhibitor of inducible nuclear import of NF-κB and other SRTFs suppresses systemic expression of proinflammatory cytokine genes and reduces LPS lethality.

EXPERIMENTAL PROCEDURES

Peptide Synthesis—The SN50 and SM peptides were synthesized, purified, filter-sterilized, and analyzed as described elsewhere (23–25). The SN50 peptide carries an NLS derived from NF-κB1 (p50), whereas the SM peptide functional motif contains NLS mutations that preclude recognition by the NLS receptor, importin α (also called karyopherin α or Rch 1) (24). The cSN50 peptide was synthesized and analyzed in a similar manner. To assure an efficient coupling of fluorescein isothiocyanate (FITC; Pierce Chemical, Rockford, IL) to cSN50, three glycine residues were added to the NH_2 terminus of cSN50. The coupling of FITC was done according to the manufacturer's manual.

Cell Line—Murine macrophage RAW 264.7 cells were obtained from the American Type Culture Collection (Manassas, VA). These cells were cultured in Dulbecco's minimal essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum containing no detectable LPS (< 0.006 ng/ml as determined by the manufacturer, Atlanta Biological, Norcross, GA), 2 mM L-glutamine, and antibiotics.

Detection of cSN50 and SN50 Peptides—Intracellular persistence of cSN50 and SN50 peptides in cultured macrophage RAW 264.7 cells was done by pulsing the cells with either peptide (5 μM) for 30 min, removing cells by centrifugation, and resuspending them in fresh MEM with 10% heat-inactivated fetal bovine serum. The cells were grown on chamber slides, and at specified time intervals (3–10 h) the cells were examined by indirect fluorescence with the rabbit antibody against epitope tag (LMP) present in both peptides as described previously (23).

Detection of FITC-cSN50 Peptide—Intracellular location of FITC-cSN50 peptide in cultured macrophage RAW 264.7 cells was done by confocal laser scanning microscopy using direct fluorescence (28). *In vivo* detection of FITC-cSN50 peptide in blood cells and in spleen cells of C57Bl/6 mice following intraperitoneal injection was done using fluorescence-activated cell sorting (FACS) analysis. Briefly, whole blood was collected from the periorbital plexus into heparin-containing tubes 20 min after intraperitoneal injection of 190 nanomoles of FITC-cSN50 peptide (0.7 mg) or equimolar concentration of FITC. White blood cell-rich fraction was prepared by differential centrifugation followed by the lysis of residual erythrocytes and analyzed by FACS. The mice were immediately sacrificed after blood collection and their spleens were excised, rinsed in PBS, and gently homogenized between two microscopic slides. The erythrocytes were removed by a brief hypotonic lysis. The spleen cells were spun down and resuspended in PBS supplemented with 5% heat-inactivated fetal bovine serum and analyzed by FACS. Separately, blood samples containing erythrocytes and the total spleen cell population (erythrocytes included) were analyzed by FACS. FACS analysis (FACSCalibur; Becton and Dickinson, San Jose, CA) was done using forward *versus* side light scatter, and green fluorescence was collected with a 530 ± 30-nanometer band pass filter.

Measurement of Inducible Nuclear Import—Where indicated, 80% confluent monolayers of murine macrophage cell line RAW 264.7 (100-mm plates with 10 ml of fresh medium) were stimulated with LPS from *Escherichia coli* 0127:B8 (Difco, Detroit, MI) at 30 ng for 30 min. Nuclear import of NF-κB in RAW 264.7 was measured by electrophoretic mobility gel shift assay using a radiolabeled κB probe (14, 15, 24). Inducible nuclear import of AP-1 and NFAT was measured in Jurkat T cells as described elsewhere (24, 25). Gels were dried onto chromatography paper and exposed to Fuji FLA 2000 imager plates (Fuji, Tokyo, Japan) for quantitation and then to Kodak Biomax MR autoradiography film (Eastman Kodak, Rochester, NY). The effects of [illegible] peptides on the nuclear import of SRTF was quantitated from the content measured in nuclear extracts from stimulated cells. The resulting value was normalized to 100% as the maximal inducible nuclear import. The value obtained in cells treated with cell-permeable peptide was similarly obtained and subtracted from 100% of inducible nuclear import to determine the percent inhibition.

Measurement of the Cytokines, TNFα and IFNγ, in Plasma—Blood samples from saphenous veins were collected in heparinized tubes at indicated times. Aliquots of plasma were kept at −80 °C until assayed for the cytokine levels using ELISA kits specific for TNFα and IFNγ (R & D Systems, Minneapolis, MN). Serial dilutions were made to determine the cytokine concentrations by comparison with the standard according to the manufacturer's instruction.

Murine Model of LPS-induced Lethal Shock—Female C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) that were 8–12 weeks old (20 g weight) were injected intraperitoneally with LPS (200 microliters, 4 mg/ml) from *E. coli* 0127:B8 (Difco). Cell-permeable cSN50, SN50 and SM peptides, or 0.8% pyrogen-free saline (diluent) were injected intraperitoneally before (30 min) and after (30, 90, 150, and 210 min and 6 and 12 h) LPS challenge. In experiments presented in Fig. 4*D*, the cSN50 peptide was not injected before LPS. Injections followed LPS challenge at 30, 90, 150, and 210 min and 6, 12, and 24 h. All injected agents were sterile and prepared in pyrogen-free saline. Animals were observed at 2-h intervals during the first 8 h, at 4-h intervals during the subsequent 16 h, and twice daily thereafter. Autopsies were performed shortly after death. Surviving animals were observed for 3 days in most experimental groups or for 10 days in the groups shown in Fig. 4, *B* and *D*, after which they were sacrificed and autopsied. Formalin-fixed, paraffin-embedded sections of the liver, spleen, lungs, and kidneys were stained with hematoxylin and eosin to assess overall histology. Animal handling and experimental procedures were performed in accordance with the American Association of Accreditation of Laboratory Animal Care guidelines and approved by the Institutional Animal Care Committee.

RESULTS

Inhibition of NF-κB by Linear and Cyclic SN50 Peptides in Cultured Murine Macrophages—A cell-permeable cyclic peptide termed cSN50 was designed by inserting two cysteines flanking the NLS motif of the p50-NF-κB1 to form an intrachain disulfide bond (Table I). In addition, FITC-conjugated cSN50 peptide (FITC-cSN50) was synthesized to monitor its intracellular location in cultured cells *in vitro* and in blood and spleen cells *in vivo*. As shown in Fig. 1*A*, fluorescence confocal laser scanning microscopy of cultured murine RAW 264.7 macrophages demonstrated intracellular accumulation of the FITC-cSN50 peptide. This finding is concordant with prior studies of its linear analog, SN50 (23). The cytoplasmic localization of FITC-cSN50 peptide is consistent with the intracellular distribution of its target, importin α (karyopherin α), in non-stimulated cells (24). When intracellular persistence of cSN50 and SN50 peptides was analyzed using indirect fluorescence (see "Experimental Procedures") no detectable differences were observed (data not shown).

We next assessed whether the cSN50 peptide inhibits the nuclear import of NF-κB in cultured murine macrophages. Macrophages are known targets for the proinflammatory agonist, LPS, which can induce endotoxic shock. When tested in LPS-stimulated murine RAW264.7 macrophages, cSN50 inhibited the nuclear import of NF-κB at a concentration of 10 to 30 [illegible] for SN50

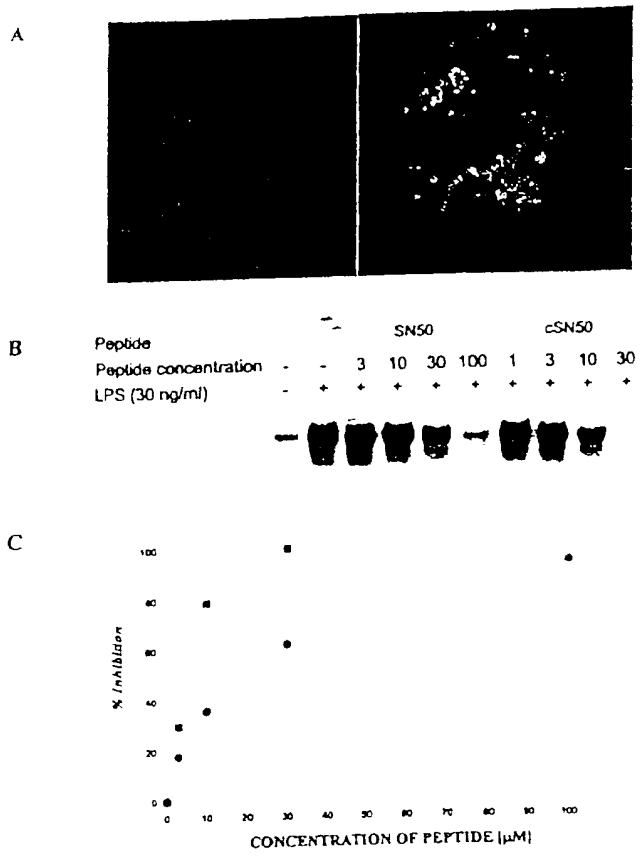

FIG. 1. **Intracellular delivery and inhibitory activity of the cSN50 peptide toward NF-κB nuclear import in cultured cells.** *A*, fluorescence confocal laser scanning microscopy of murine macrophage RAW 264.7 cells incubated with 5 nanomoles of FITC-cSN50 peptide (*right panel*) or with a similar concentration of unconjugated FITC (*left panel*). Midcell, the 1-μm section demonstrates intracellular location of FITC-cSN50 peptide. *B*, inhibition of LPS-induced NF-κB nuclear import in murine macrophage RAW 264.7 cells by SN50 and cSN50 peptides. The peptides were added to cells at concentrations shown 20 min before addition of LPS. Nuclear extracts were analyzed using a radiolabeled κB consensus sequence (23–25). Note greatly reduced radioactivity in SN50 and cSN50-treated cells, indicating inhibited nuclear import of NF-κB, as compared with controls. *C*, comparison of the *in vitro* inhibitory potency of SN50 and cSN50 peptides. Gels described in the legend to Fig. 1*B* were dried and exposed to Fuji FLA 2000 Imager plates for quantitation. Each data point represents mean value (S.D.) of three independent experiments. *Solid circles* represent SN50 peptide, and *solid squares* denote cSN50 peptide. For details see "Experimental Procedures."

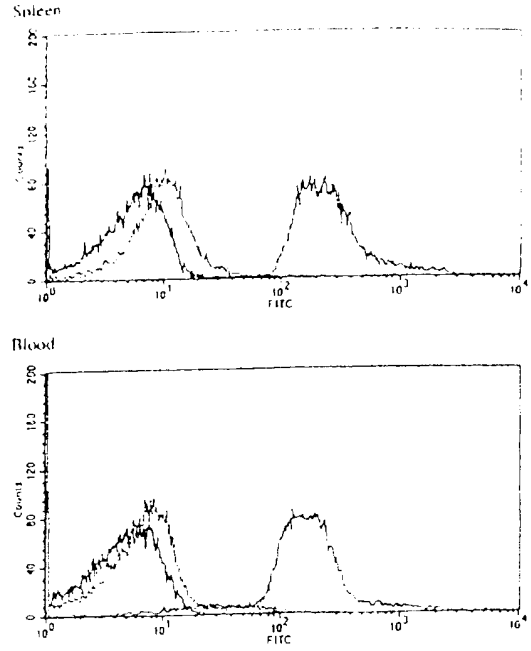

FIG. 2. ***In vivo* delivery of the cSN50 peptide.** FACS analysis of leukocytes and lymphocytes isolated from whole blood of mice (*upper panel*) and spleen cells (*lower panel*) 20 min after intraperitoneal injection of 190 nanomoles of FITC-cSN50 peptide (*green*), 190 nanomoles of unconjugated FITC (*red*), or saline (*black*).

tively expressed nuclear CCAAT-binding factor, NF-Y, remained unchanged (results not shown). Based on quantitative phosphoimager analyses of these experiments (n = 3) presented in Fig. 1*C*, cSN50 was 3 times more potent than the linear SN50 peptide (IC_{50} = 7 *versus* 21 μM). The cSN50 peptide was 3–10 times more inhibitory than the SN50 peptide toward the nuclear import of AP-1 and NFAT, respectively, when analyzed in Jurkat T cells stimulated with phorbol ester and ionomycin (results not shown). These experiments with the cultured cells provided a basis for the *in vivo* delivery of cSN50 to examine its effect on the expression of cytokine genes and the outcome of LPS-induced lethal shock.

Delivery of cSN50 Peptide to Blood and Spleen Cells in Vivo—To determine whether cell-permeable cSN50 peptide injected intraperitoneally is delivered to blood cells and to the spleen, peripheral blood leukocytes and lymphocytes, as well as isolated spleen cells, were analyzed by FACS. As shown in Fig. 2, a leukocyte/lymphocyte-rich fraction prepared from blood of mice 20 min after intraperitoneal injection of FITC-cSN50 (0.7 mg) showed its transfer to circulating white blood cells based on a strong fluorescence signal detectable in examined cells (green tracing). Leukocytes and lymphocytes prepared from the blood of control mice injected with unconjugated FITC produced a background signal (red tracing) similar to that observed in leukocytes and lymphocytes isolated from the blood of animals receiving intraperitoneal saline injection (black tracing). Monitoring the delivery of FITC-cSN50 peptide to spleen cells, predominantly T and B lymphocytes, showed a strong fluorescence gain as compared with that measured in splenocytes obtained from mice injected with unconjugated FITC or saline (Fig. 2). FITC-cSN50 peptide was also detectable in other blood cell fractions, including red blood cells, analyzed by FACS (results not shown). There was no detectable hemolysis in blood samples collected from cell-permeable peptide-treated animals. These results indicate that FITC-cSN50 peptide injected intraperitoneally was rapidly delivered to circulating blood cells and to the spleen. This type of analysis cannot establish what fraction of intraperitoneally injected FITC-cSN50 peptide was delivered to blood and spleen cells.

cSN50 Peptide Suppresses Cytokine Expression in Vivo—Injection of LPS into humans, primates, or mice leads to an early burst of proinflammatory cytokine mediators of endotoxic shock such as TNFα, IL-1, and IFNγ (29–35). To test whether *in vivo* delivery of cSN50 will attenuate expression of cytokine genes regulated by NF-κB and other SRTFs, we injected C57BL/6 mice intraperitoneally with 800 μg of LPS from *E. coli* serotype 0127:B8 (LD_{100} = 800 μg). Consistent with prior studies (36), mice challenged with LPS showed an early burst of TNFα expression at 1–2 h, followed by increased IFNγ production peaking at 24 h (Fig. 3). In contrast, there was no rise in TNFα expression in the cSN50 peptide-treated mice (1.5 mg given intraperitoneally in 7 injections 30 min before to 12 h after LPS challenge). This effect was statistically significant ($p < 0.004$) using the area under the curve method and the Student's t test (37). Likewise, the more progressive rise of

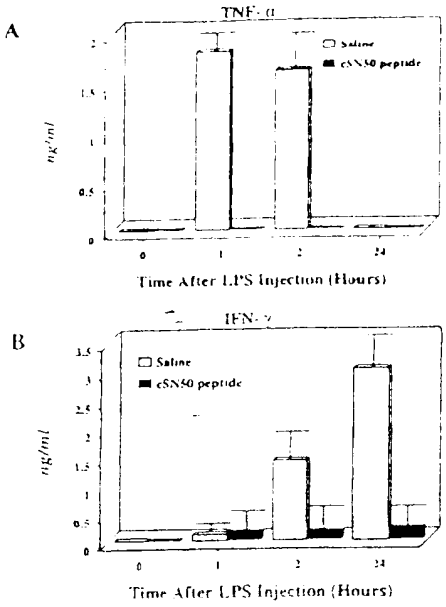

FIG. 3. *In vivo* levels of proinflammatory cytokines, TNFα and IFNγ, in LPS-challenged mice following administration of saline (*open bars*) and cSN50 peptide (*solid bars*). Female C57Bl/6 mice (20 g) were randomly grouped (3 mice per treatment group) and received intraperitoneal injection of LPS (*E. coli* 0127:B5, 800 μg). The cSN50 peptide (1.5 mg) was injected intraperitoneally at 30 min before LPS and afterward at 30, 90, 150, and 210 min and 6 and 12 h. Pyrogen-free saline (diluent) was injected into control mice. At indicated time intervals, blood samples were obtained, and cytokine levels were measured by ELISA in heparinized plasma. All mice receiving saline died within 72 h, and those treated with the cSN50 peptide survived. The bars represent mean ± S.D.

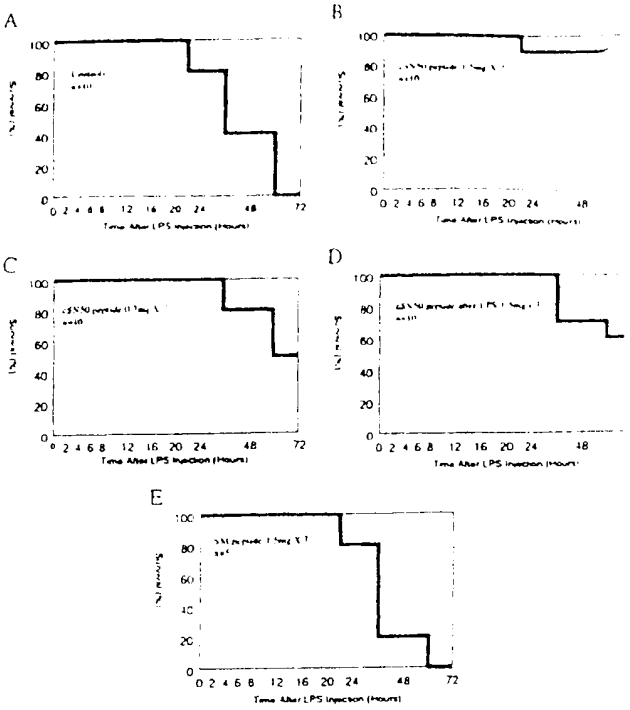

FIG. 4. Survival of control and peptide-treated mice chal lenged with LPS. Female C57Bl/6 mice (20 g) were randomly groupe (10 mice per group). Treatments included cSN50 (1.5 or 0.7 mg) and SN peptide (1.5 mg) given 30 min before LPS (*E. coli* 0127:B5, 800 μg) an afterward at 30, 90, 150, and 210 min and 6 and 12 h. *A*, controls, saline *B*, cSN50 peptide, 1.5 mg × 7; *C*, cSN50 peptide, 0.7 mg × 7; *D*, n peptide before LPS and cSN50 peptide (1.5 mg × 7) given 30 min af LPS and at 90, 150, and 210 min and 6, 12, and 24 h; *E*, SM peptide mg × 7.

tide ($p < 0.04$). Thus, *in vivo* delivery of cSN50 resulted in a striking attenuation of expression of genes encoding two key proinflammatory mediators of endotoxic shock, TNFα and IFNγ. This is consistent with prior *in vitro* data on SN50 peptide-induced attenuation of expression of genes encoding IL-2, Fas ligand, and COX 2 measured as mRNA transcripts or protein levels in cultured cells (24, 26, 27).

In Vivo Delivery of cSN50 Peptide Prevents LPS-induced Lethal Shock—Almost complete *in vivo* suppression of the key cytokine mediators of endotoxic shock by cSN50 correlated with a reduction in mouse lethality. As shown in Fig. 4*A*, all LPS-injected animals died within 72 h. In contrast, mice treated with cSN50 (1.5 mg given in 7 injections 30 min before to 12 h after LPS challenge) were essentially protected throughout the 72-h period, as evidenced by the cumulative survival rate of 90% (Fig. 4*B*). Survivors observed for the subsequent 10 days showed no apparent signs of disease. This protective effect of cSN50 was reduced (50% survival) when the dose was decreased to 0.7 mg per injection (Fig. 4*C*). To determine whether the cSN50 peptide reduces lethality when administered *after* exposure to LPS, the first peptide dose was given 30 min after endotoxin. The survival rate was 60%, indicating that the cSN50 peptide attenuates endotoxic shock, if given shortly after exposure to LPS (Fig. 4*D*). The *in vivo* protective effect was lost if the functional NLS motif was mutated, as in the SM peptide. Mutations in the NLS motif preclude recognition by the NLS receptor, importin α (also called karyopherin α or Rch 1) (24). All SM peptide-treated mice died within 72 h (Fig. 4*E*). Based on the log rank test (38), the difference in survival rate between cSN50 peptide-treated and control mice was statistically significant ($p < 0.001$). The cSN50 peptide appears to be at least 3 times more potent *in vivo* than its linear analog, LPS challenge; results not shown). This is consistent with th differences in inhibitory potency in cultured cells (Fig. 1C Cumulatively, *in vivo* data obtained with the cSN50 peptid indicate that this inhibitor of nuclear import of NF-κB an other SRTFs is effective in attenuating systemic expression c proinflammatory cytokine mediators of endotoxic shock and it lethal outcome.

DISCUSSION

When delivered to cultured cells, the cell-permeable cycli peptide, cSN50, inhibits inducible nuclear import of NF-κB an other SRTFs. We have demonstrated not only *in vitro* deliver of cSN50 to murine macrophages but *in vivo* targeting of thi new cyclic peptide to blood and spleen cells in mice. Accorc ingly, we tested whether the *in vivo* delivery of cSN50 modifie the expression of proinflammatory cytokines and lethal shoc in response to LPS. These *in vivo* experiments demonstrate that cSN50 attenuates expression of genes encoding two ke mediators of endotoxic shock, TNFα and IFNγ, as well a provides a highly significant protection of mice from LPS-ir duced lethal shock. Remarkably, *in vivo* delivery of cSN50 at it optimal concentration resulted in a 90% survival rate amon mice receiving LD_{100} of LPS.

As expected from studies of SN50 (24), its cyclic analo cSN50, inhibits nuclear import of NF-κB and other SRTF However, cyclization of SN50 appears to enhance its inhibito effects toward NF-κB signaling. The enhanced inhibitory tency of cSN50 does not appear to be due to its longer intrace lular persistence than that of SN50 peptide. Rather, it ma reflect a higher affinity of cSN50 *versus* SN50 for importin (karyopherin α), the intracellular target of these peptides (24

nuclear transcription factor, NF-Y, suggesting that the peptide preferentially affects inducible nuclear import. Based on prior studies with SN50 peptide, we postulate that its intracellular target is most likely the cytoplasmic NLS receptor, Rch 1 (importin α/karyopherin α) (24). The broad inhibitory range of cSN50 toward the nuclear import of NF-κB and other SRTFs is an advantage rather than a drawback to its *in vivo* delivery for the following reasons. First, the cytokine genes that mediate systemic inflammatory response are regulated by multiple SRTFs. In particular, the gene encoding TNFα is regulated by NF-κB, NFAT, and ATF2/Jun, whereas the gene encoding IFNγ is controlled by NF-κB and NFAT (7–9). Almost total suppression of TNFα and IFNγ expression by cSN50 in mice challenged with LPS indicates that *in vivo* inhibition of SRTF-regulated gene transcription was achieved. Second, the requirement for repeated *in vivo* administration of cSN50 may reflect the time-limited intracellular inhibitory activity of cell-permeable peptides (Refs. 22 and 24 and data not shown). Hence, its *in vivo* inhibitory effect on inducible genes expression regulated by SRTFs is reversible. On the other hand, the *in vivo* kinetics of TNFα and IFNγ induction differs (see Fig. 3), thereby requiring the maintenance of *in vivo* delivery of cSN50 peptide over the 12-h period. Third, the *in vivo* inhibitory effect of cSN50 peptide was dependent on the presence of a functional NLS. Mutations that inactivate NLS function yield a cell-permeable peptide (SM) that fails to affect the acute systemic inflammatory response to LPS in this experimental model of lethal shock. Fourth, the cSN50 peptide exhibited no detectable side effects when administered to control animals in the absence of LPS (results not shown). Taken together, these findings suggest that an *in vivo* delivery of cSN50 is functionally effective and safe.

In summary, these experiments indicate that cSN50 can be delivered *in vivo* to cells involved in synthesis of proinflammatory cytokines. In turn, the expression of genes encoding these key mediators of endotoxic shock is suppressed, resulting in reduced mortality. Although endogenous inhibitors of cytokine signaling, such as members of the CIS/SOCS/JAB/SSI family, negatively regulate signal transduction (39), all exogenous inhibitors of LPS toxicity tested *in vivo* and reported until now are targeted toward the interaction of LPS or cytokines with their cognate receptors (29–32, 34, 40, 41). In contrast, the cell-permeable peptides described here are targeted intracellularly (22). As a reversible inhibitor of the nuclear import of SRTFs, cSN50 represents a new class of anti-inflammatory agents capable of suppressing systemic inflammatory responses *in vivo*. In keeping with this concept, SN50 is protective when delivered to mice challenged with a low dose of LPS in combination with D-galactosamine,[2] which renders mice hypersensitive to LPS (42). In addition, preliminary experiments indicate that *in vivo* delivery of the cSN50 peptide is effective in blocking lethal shock induced by the superantigen, staphylococcal enterotoxin B, that robustly activates a subset of T cells (data not shown).

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[2] X. Y. Liu, D. Robinson, R. A. Veach, S. Timmons, R. D. Collins, and J. Hawiger, unpublished observations.

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